PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Peilin Chen et al.

Application No.: 10/650,595

Filed: August 27, 2003

For: MULTICONSTITUENT LIQUID IGG AND IGM CALIBRATORS

Customer No.: 20350

Confirmation No. 7928

Examiner: CHEN, Stacy Brown

Technology Center/Art Unit: 1648

DECLARATION UNDER 37 C.F.R. §1.132 OF DR. PEILIN CHEN

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Peilin Chen, being duly warned that willful false statements and the like are punishable by fine or imprisonment or both (18 U.S.C. § 1001), and may jeopardize the validity of the patent application or any patent issuing thereon, state and declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I received a Ph.D. in Medicinal Chemistry from China Pharmaceutical University in 1989. I served as a Senior Scientist III at Metrika from 1998 to 1999. Currently, I

am a Senior Staff Scientist at Bio-Rad Laboratories, and have been at this and related positions for over 6 years. A copy of my curriculum vitae is attached as Exhibit A.

3. The invention of the above-referenced patent application provides, for the first time, a multiconstituent liquid IgG and IgM calibrator that can be used as a positive control, or a calibrator, in multi-analyte immunoassays. This claimed calibrator contains a plurality of antibodies that are dissolved in a serum. These antibodies specifically recognize at least ten different antigens derived from microorganisms including *Toxoplasma gondii*, Rubella virus, Cytomegalovirus (CMV), Herpes Simplex Virus type-1 (HSV-1), Herpes Simplex Virus type-2 (HSV-2), Mumps Virus, Measles Virus, Epstein-Barr Virus (EBV), Varicella Zoster Virus, *Borrelia burgdorferi*, *Treponema pallidum*, *Helicobacter pylori*, and *Mycoplasma pneumoniae*.

4. I am a named inventor on this patent application. I have read and am familiar with the contents of this patent application. In addition, I have read the Office Action mailed February 1, 2005, and the Final Office Action mailed July 26, 2005, for the present application. It is my understanding that the Examiner believes that the present invention is obvious over the Wong reference (U.S. Patent No. 5,478,753), which describes a calibrator that contains antibodies with immune specificity against four different pathogenic organisms.

5. This declaration is provided to establish that, while it is relatively easy to construct a calibrator that is designed for individual immunological assays and contains antibodies for up to four or five distinct antigen specificities, it is increasingly difficult to include additional antibodies of different antigen specificities in the calibrator for a multiplexing immunoassay, due to the instability of an antibody solution at a high total antibody concentration, as well as the dilution effect caused by mixing multiple sera containing antibodies of desired specificities. When one combines ten or more different antibodies, there is no reasonable expectation of success that a functional calibrator will be produced.

6. Antibodies are relatively large glycoproteins that consist of two light chains and two heavy chains. The average molecular weight of a monomer human IgG antibody is about 150 kDa, whereas a pentamer human IgM is about 950 kDa. Synthetic antibodies, such

as those used in the calibrator of the Wong reference, have an average molecular weight of over 1,000 kDa. Through the variable regions located near the N-terminus of the light and heavy chains, antibodies bind with specificity to their respective antigens. Through the constant regions near the C-terminus of the heavy chains, antibodies bind with other proteins such as Fc receptors and components of the complement pathway. Because of their high molecular weight and their propensity to bind to various other molecules, antibodies are more likely to form aggregates and/or precipitate from a solution when their combined concentration reaches a certain level.

7. When one starts with a calibrator containing four or five different antibodies, each present at a level that is sufficient to allow detection through its complex with its corresponding antigen, to include additional antibodies into the calibrator, it becomes more and more difficult as the number of the antibodies increases. As these additional antibodies are introduced into the serum, each of such additional antibodies must also reach a level that is sufficient for detection through antigen-antibody complexing. To ensure that the antibodies remain dissolved in the serum, these antibodies combinedly must also remain below the concentration where antibody aggregates form or antibodies precipitate from the solution and the resulting composition becomes non-functional as a calibrator.

8. On the other hand, the difficulties of constructing a multi-antibody calibrator relate to the dilution effect caused by mixing multiple sera containing antibodies of desired specificities. When antibodies present in multiple sources (*e.g.*, multiple sera) are simply mixed, especially when the number of antibodies is large (*e.g.*, ten or more), each of the antibodies will inevitably become overly diluted and fall below the necessary concentration for detection. Thus, there is no reasonable expectation to successfully construct a functional calibrator containing ten or more different antibodies.

9. Despite the difficulties in constructing a calibrator containing antibodies that have ten or more different antigen specificities and remain dissolved in a serum, the present inventors have in fact successfully assembled several IgG and IgM calibrators, each of which containing antibodies with specificities against more than ten different antigens derived from

microorganisms including *Toxoplasma gondii*, Rubella virus, Cytomegalovirus (CMV), Herpes Simplex Virus type-1 (HSV-1), Herpes Simplex Virus type-2 (HSV-2), Mumps Virus, Measles Virus, Epstein-Barr Virus (EBV), Varicella Zoster Virus, *Borrelia burgdorferi*, *Treponema pallidum*, *Helicobacter pylori*, and *Mycoplasma pneumoniae*. These calibrators have been shown in actual testing to be fully functional as calibrators or positive controls for multi-analyte immunological assays. This is the first time that anyone has successfully produced a useable calibrator containing antibodies with more than ten antigen specificities.

10. In summary, because of the difficulties and uncertainty involved in constructing an immunoassay calibrator containing a plurality of antibodies of ten or more different antigen specificities, there is no reasonable expectation that a functional multi-specificity calibrator can be readily made. The multi-antibody composition of the present invention is therefore not obvious in view of the Wong reference.

Date: November 10, 2005 By: Peilin Chen

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PROFILE

- R&D scientist specialized in immunoassay development, organic/peptide synthesis and protein chemistry with over five years experience in supervising other scientists and project management.
- Consistently recognized by executive management and colleagues for cross-discipline talents in needs analysis, troubleshooting and problem resolution in fast-paced technical environments.
- Effectively plan and conduct technical presentations in a professional manner for executive audiences; research and prepare reports and articles for publication.

SELECTED ACHIEVEMENTS

- Successfully developed a multiplexed serological IgG assay of 15 infectious disease antibodies, a multiplexed serological IgM assay of 12 analytes and a multiplexed thyroid assay including third generation TSH, FT4, FT3, Anti-TPO and Anti-Tg assay on the Bio-Rad BioPlex 2200 immunoassay analyzer.
- Directed development of custom peptide synthesis technologies generating $2 millions in sales within the first two years of a start up company.
- Ten publications, ten abstracts and four patent disclosures.

TECHNICAL EXPERTISE

***In Vitro* Diagnostics**
- Assay development from concept to validation (Strip assay, ELISA, Flow Cytometry, Automatic Immunoassay Analyzer)
- Multiplex technology (Luminex)
- Antigen and antibody coupling chemistry onto variety of solid phases
- Hapten design and synthesis
- Antibody purification and digestion
- Protein and happen conjugation, scale up and conjugate characterization
- Familiarity with Design Control and cGMP

Protein Chemistry
- Purification and characterization
- Chromatography (gel filtration, ion exchange, HIC, affinity purification)
- Electrophoresis and western blot
- Mass Spectrometry (MOLDI-TOF)

Medicinal/Organic/Peptide Chemistry
- Molecular (drug) design and synthesis
- Structure-activity relationship
- Peptide synthesis (solution and solid phase) and libraries
- Interpretation of molecular structure via NMR, MS, and IR
- TLC and flash chromatography
- HPLC

EXPERIENCE

BioPlex Division, Bio-Rad Laboratories, Hercules, California, Oct. 1999-Present

Senior Staff Scientist **July 2005 -Present**

Lead Biological program to develop critical biological materials used in the Bio-Plex immunoassays supporting launch of the Bio-Plex 2200 Immunoassay analyzer, also manage custom antibody programs

Group Leader **July 2003 – June 2005**

Supervised a group of five scientists, interviewed and hired qualified personnel and managed multiple projects including serology IgG, serology IgM, thyroid and engineered antibody projects

- Developed multiplexed ToRCH IgM and IgG kits on the Bio-Rad BioPlex 2200 Immunoassay Analyzer
- Developed a multiplexed Thyroid assay including third generation TSH, FT4, FT3, anti-TPO and anti-Tg
- Headed and coordinated projects with consultants, research associates and scientists both within Bio-Rad worldwide and with outside organizations
- Developed methods to prepare multiconstituent thyroid calibrators (5 analytes)
- Wrote numerous project plans, reports and abstracts, and presented project updates at different meetings in Bio-Rad and the abstracts at scientific meetings

Team Leader and Staff Scientist **July 2000 – June 2003**

Primary responsibilities including developing a multiplex serology IgM assay on the Bio-Plex 2200 immunoassay analyzer, assigning daily work to the team and managing the project to meet timelines set by the management

- Completed feasibility and predevelopment of serology IgM assay for detecting 12 infectious IgM antibodies (Toxoplasma, Rubella, CMV, HSV1, HSV 2, Syphilis, Lyme, EBV VCA, Heterophile, Mumps, Measles, and VZV)
- Developed methods to prepare multiconstituent serology IgM calibrators (12 analytes)
- Completed serology IgM feasibility report, subsequently approved by Design Review Board of the company
- Filed four patent disclosures

Senior Scientist **October 1999 – June 2000**

- Proposed and developed type-specific HSV 2 IgG assay
- Successfully conducted a feasibility study of a multiplexed serological IgG assay including 15 infectious disease antibodies on Luminex 100. The antibodies are to *Toxoplasma gondii*, Rubella, CMV, HSV 1, HSV 2, Lyme, Syphilis (3), EBV VCA, EBV EAD, EBV EAR, EBV NA, Mumps, Measles and *H. pylori*,
- Developed methods to prepare multiconstituent serology IgG calibrators(15 analytes)

Diagnostic Reagents Inc., Sunnyvale, California

Consultant **April 1999 - October 1999**

- Synthesized haptens of Gentamicin, Tobramycin and Hyhrochlorothiazide
- Developed HPLC method for detection of Chromate (IV) in urine samples
- Worked with a team to develop a method for detecting adultery samples, such as Urea Luck, in TDM tests

Metrika, Inc., Sunnyvale, California

Senior Scientist III **June 1998 – August 1999**

Led a high sensitivity project for improvement of quantitative immunochromatographic assays compatible with Metrika's DRx (Digital Response) diagnostic device, using fluorescence and fluorescence resonance energy transfer (FRET) technology and worked with a cardiac team to resolve technical issues.

- Transformed Metrika's DRx platform from reflectance-based measurement to a fluorescence detection system and developed a quantitative, single-use miniaturized fluorescence immunoassay device
- Demonstrated the feasibility of fluorescence-based lateral flow hCG assay with a range of 5-20,000 mIU/ml in plasma and whole blood on a single strip with clinical precision of < 10% using the above DRx fluorescence immunoassay device
- Assembled a modular instrument capable of measuring absorbance, reflectance, fluorescence, FRET, chemiluminescence, either in solution or on immunoassay strips
- Scaled up a conjugation process up to 600 mg

Biocircuits Corporation, Sunnyvale, California

Scientist II **May 1996 – June 1998**

Responsible for developing FT4 assay from concept and design specification to reagent evaluation, process optimization, transfer and verification

- Designed and synthesized new thyroxine haptens and conjugates, and successfully developed free thyroxine assay in dry chemistry format. The 510 (K) clearance of free thyroxine assay on the IOS immunoassay system was received from FDA in April, 1998 (37 days review cycle).
- Developed and modified conjugation methods for scaling up to improve yields and efficiency. Transferred those processes to manufacturing department
- Documented the product design history for free thyroxine assay and wrote new protocols related to the assay
- Improved T4/T4 Uptake assay performance by changing chemical structure of the hapten on the conjugate
- Designed and characterized conjugates to support TSH, PSA, hCG and digoxin assay development.

AnaSpec Inc., San Jose, California

Technical Director **February 1995 – May 1996**

Directed the development of technologies in custom peptide synthesis generating $2 millions in sales within the first two years for the start-up company

- Developed strategies and technology for synthesis and purification of a 200 g scale of peptides
- Improved the output of custom peptide synthesis by modification of analytical and preparative HPLC methods and workflow

Scientist **June 1994 – February 1995**

- Synthesized a variety of peptides using both solution and solid phase methodology
- Synthesized a variety of fluorogenic and chromogenic peptide substrates

College of Pharmacy, University of Kentucky, Lexington, Kentucky

Postdoctoral Scholar **September 1989 - May 1994**

- Designed and synthesized various haptens for production of polyclonal and monoclonal antibodies and developed immunoassays for detection of drugs. The technology was applied in a start-up

company ultimately acquired by Neogen Corporation

- Designed and synthesized transitional state analogs of cocaine for production of catalytic antibodies
- Improved sensitivities of ELISAs by modifying the structures of hapten-enzyme conjugates.
- Totally synthesized haptens of myo-inositol 1, 4, 5-triphosphate
- Synthesized several novel reagents for photoaffinity labeling of receptors and enzyme active sites; studied on 15-hydroxyprostaglandin dehydrogenase, glutamate dehydrogenase, cyclosporin A binding protein and sigma receptor
- Synthesized and radiolabeled several peptidyl carbamate inhibitors for elastase and improved the synthetic procedure
- Synthesized and evaluated the stability of phenylbutazone prodrugs and developed analytical methods with HPLC to identify the prodrugs

EDUCATION

College of Pharmacy, University of Kentucky, Lexington, **Postdoctoral Scholar**, Medicinal Chemistry, 1994
China Pharmaceutical University, Nanjing, China., **Ph.D.,** Medicinal Chemistry, 1989
China Pharmaceutical University, Nanjing, China, **M.S.,** Medicinal Chemistry, 1986
China Pharmaceutical University, Nanjing, China, **B.S.,** Medicinal Chemistry, 1982

PUBLICATIONS

1. Kaul, R., **Chen, P.**, and Binder, SR. (2004) Detection of immunoglobulin M antibodies specific for *Toxoplasma gondii* with increased selectivity for recently acquired infections. J. Clin. Microbiol. 42(12): 57-5-5709
2. **Chen, P.,** Tian, Z., Digenis, G.A. and Tai, H.H. (1996) Enzyme immunoassay of two nicergoline metabolites, 10α-methoxy-9,10-dehydrolysergol (MDL) and 1-methyl-10α-methoxy-9,10-dehydrolysergol (MMDL). Res. Commun. Mol. Path.Pharm. 92 (3): 315-328
3. **Chen, P.,** and Tai, H.ll. (1995) A sensitive enzyme immunoassay for cyclosporin A using antibodies generated against a novel hapten. Res. Commun. Mol. Path. Pharm.88(3): 317-326
4. **Chen, P.,** Ensor, C.M. and Tai, H.H. (1995) A [^{125} I]-labeled N6 -substituted azido analog Of NAD+ for photoaffinity labeling of NAD+ -linked enzymes. Photochem. Photobiol. 60: 445-458
5. **Chen, P.,** Watt, D.S. and Tai, H.H. (1994) Improved sensitivity of enzyme immunoassay for cocaine and benzoylecgonine using heterologous hapten-enzyme conjugates. Res. Commun. Subst. Abuse 15: 71-82
6. **Chen, P.,** Hussian, A. and Tai, H.H. (1994) An improved method of radioiodination with chloramine T. Anal. Biochem. 219:159-161
7. Digenis, GA., McClanaham, J.S. and **Chen, P.** (1993) The synthesis of [2,3,4,5,-^{14}C]-l-vinyl-4-pyrrolidinone. J. Labeled Compd. Radiopharm. 27(12): 11-17
8. **Chen, P.,** Peng, S.X. and Yang, Z.X. (1992) Synthesis and biological activity of some Val (Ala)-Tyr and Val-Tyr-Tyr peptides. Yaoxue Xuebao. 27(12): 895-903
9. Yue, C.G., **Chen, P.** et al. (1988) Synthesis of podophyllotoxin derivative: podophylloylethyl hydrazine (SP-1). Qansu Yaoxue. 3(2): 9-12
10. **Chen, P.,** Peng, S.X. and Yang, Z.X. (1987) Synthesis of N-(mercaptobenzoyl)-N-(alkyl/aryl)-glycines and the corresponding disulfides. Yaoxue Xuebao. 22(9): 662-670

ABSTRACTS

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2. Yu, K., S. Binder, **P. Chen**, C. Hixson, F. Torres, S. Garcia, J. Wang. A multiplex syphilis IgM serological test for the diagnosis of Treponema pallidum infection. Clin. Chem. 50(S6):A157,2004
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4. **Chen, P.,** R. Ashley-Morrow, S. R. Binder, G. Marr, C. Hixson, R. Bruehl, P. Ruiz, S. Garcia, H. Yu, Felipe Torres, and L. Le. Multiplexed Serology Test for Detection of Herpes Simplex Virus Type-Specific Immunoglobulin G Antibodies Using the Bio-Rad BioPlex 2200 Immunoassay Analyzer. ASM, New Orleans, LA. V018, 2004
5. Binder, S., **P. Chen**, L. Cuadra, J. Goodrich, L. Le, G. Marr, R. del Rosario, F. Torres, H. Yu, J. Wang. Development of a multiplex composite Treponema pallidum IgG and IgM test on the Bio-Rad BioPlex 2200. ASM, New Orleans, LA. V027,2004
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9. **Chen, P.**, S.R. Binder, C. Hixson, S. Garcia, M.B., Portella, R Kaul, and J. Wang, Multiplexed Serology Test for the Determination of Acute Infectious Mononucleosis. Clin. Chem. 49(S6):A135,2003
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